A SYNOPSIS OF THE THESIS
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"Studies on Biological Control of Post Harvest Diseases of Mango, Grape and Banana Fruits in Marathwada Region, Maharashtra."

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ABSTRACT:

India is the second largest producer of fruits in the world, contributing 11% of the total production. A main limiting factor for the commercial production of fruits is their susceptibility to post harvest pathogens. Biological control and use of botanicals to control these post-harvest diseases has gained importance due to recent global awareness on side effects of chemical agents. The present endeavor is an attempt to isolate, identify different post-harvest pathogens of fruits and to identify newer biological control agents and methods against these pathogens.

1. Introduction:

As fruits contain all the required nutrients for a balanced diet, they are the essential items in everyday meals. Fruits have medicinal and aesthetic value too. India is considered as the fruit basket of the world accounts for 11 per cent of the world's fruit production. It is the largest producer of mangoes, bananas, papaya, sapota, chillies, ginger and okra etc (Neeraj et al. 2017).

In Marathwada region, various types of fruit crops are cultivated *viz.* banana, mango, grape-vine, pomegranate, guava, sputa, Jamul, zuzuba etc. Fruits are important part of the human diet, because they supply essential nutrients such as vitamins, minerals and they are important to human health and well-being for their contents in antioxidants and anticancer substances. An increasing awareness by consumers that diet and health are linked resulted in a greater consumption of fruit. At the same time, consumers are also more concerned about the safety of the food they eat and they ask for food free from pesticide residues, toxins and pathogens.

During storage, a number of fungi are known to cause spoilage of fruits. Under tropical conditions considerable losses are occurred due to rots caused *by Rhizopus sp*., *Penicillium sp., Aspergillus sp., Botrytis cinerea, Botryodiplodia sp., Bipolaris sp., Curvularia sp., Fusarium sp.,* and are becoming limiting factor for successful fruit harvest management (Thomas, 1986). The total estimated losses of fruits and vegetables in India due to inadequate post-harvest handling, transportation and storage are reported to the extent of 20-25 percent (Singh, 1981). These losses in terms of monetary value are more than 1.5 billion dollars annually. Post-harvest losses caused by microorganism accounts for millions of dollars in perishable produce

every year (Narayanaswamy, 2005). To minimize these losses and the production, it is very necessary to control the post-harvest diseases. Post-harvest diseases can be controlled by biological, chemical and physical treatments (Mehrotra et al., 1998). These includes the use of bioagents and botanicals as biological treatments while chemical treatments includes use of antibiotics, fungicides, oils and as physical treatments vapor emitting compounds. Use of probiotics for the control of post-harvest diseases has opened new avenue in the management of post-harvest plant pathogens and organic farming. Such probiotics has never been used in the crop production or crop protection programme in agriculture. The post-harvest pathogens particularly Rhizopus, Aspergillus, Penicillium and Alternaria can be checked as a post-harvest pathogen by sprays of probiotics under natural field conditions when the load of inoculums of these post-harvest pathogens is less in fruits.

Banana "*Musa paradisiaca L.*" is one of the most popular fruits in India. In a survey carried out in Allahabad fruit markets, six fungal pathogens viz., *Fusarium moniliforme*, *Alternaria alternate*, *Aspergillus flavus, Curvularia lunata, Penicillium sp.* and *Cladosporium sp.* were found associated with postharvest diseases of banana.

Papaya (*Carica papaya L.*) is grown worldwide in the tropics and subtropics. One of the major reasons of post-harvest losses of papaya production is fungal diseases. Post-harvest disease organisms of papaya are *Rhizopus stolonifer*, *Alternaria alternate* (Robert,1997). Probiotics are defined as live microorganisms that beneficially affect the host by improving its intestinal microbial balance.

Mango (*Mangifera indica L.)* is an important fruit crop from Anacardiaceae family. Anthracnose disease of mango is one of the major pre- and post-harvest diseases of mango fruit throughout the world and also in Bangladesh which is caused by *Colletotrichum gloeosporioides*. Anthracnose attacks flowers, young fruits, leaves and twigs, even this disease can also appear in the storage of mature fruits. Disease symptoms appear as slightly, black, sunken irregular shape lesions, which gradually enlarge.

2. Review of Literature and Development in the subject

Sangeetha and Rawal (2008) reported that Anthracnose caused by *Colletotrichum*

gloeosporioides is one of the most important diseases of mango. Heavy sporulation was observed where maltose was used as carbon source followed by moderate sporulation in fructose and lactose. Among the different nitrogen sources tested, ammonium nitrate supported good growth and sporulation. Potassium nitrate and sodium nitrate also showed good growth but with moderate sporulation.

Rahman et a. (2008) a total of seven fungi were identified from the surface of fully matured papaya fruits. They were identified as *Botryodiplodia theobromae*, *Colletotrichum capsici, C. gloeosporioides, Fusarium sp., Phomopsis sp., Rhizopus stolonifor* and *Stemphylium sp.*

Liu (1986) reported that Anthracnose (*Colletotrichum sp.)* is one of the main disease causing decay and commercial devaluation of harvested mangoes. He suggested that pathogenic fungi such as anthracnose on mango fruit are latent and a main cause of serious decay.

Deka et al. (2006) reported that the post harvest loss of banana is highest (22.00%) among all the fruits in the state of Assam. Claudia et al. (2004) reported that the fungus *Colletotrichum musae* can cause both crown rot and anthracnose. In addition, crown rot diseases may also be caused by Fusarium, Acremonium, Verticillium, and Curvularia. The two primary postharvest rots of banana fruits are crown rot and anthracnose. The diseases usually appear on ripening fruits either at the point of sale or later after purchase. *Lasiodiplodia theobromae, Thielaviopsis paradoxa, Colletrotrichum musae, C. gloeosporioides, Fusarium verticillioides* and *F. oxysporum* are few pathogens caused postharvest diseases in Banana.

Jagana et.al. (2017) identified were anthracnose caused by *Colletotrichum musae* and crown rot caused by *Fusarium semitectum.* Crown rot disease was observed only during rainy season survey in all the varieties studied but not noticed in winter or summer in any of the variety.

Ramma et al. (1999) identified that crown rot disease was the major post-harvest disease on ripening bananas in Mauritius followed by anthracnose and blossom end rot. Crown rot was found to be due to a complex of fungal pathogens, *viz*. *Fusarium sp., Colletotrichum sp., Verticilium sp* and *Cladosporium sp.*. It was found to develop rapidly during fruit ripening thereby reducing the quality and marketability of fruits.

Snowdon (1992) demonstrated that post harvest rots of watermelon caused by *Fusarium sp.*. It was also reported that good disease control in the field rarely allow *Colletotrichum orbiculare* and *Didymella bryoniae* to cause anthracnose and black rot respectively on watermelon.

Salunkhe and Desai (1984) observed that Gray Mold (*Botrytis cinerea*) rot, Green Mold *(Penicillium digitatum*) rot and *Cladosporium sp*. are the main postharvest diseases of pomegranate fruit. Gray Mold usually starts at the calyx. Heart rot is another disorder that may be caused by *Aspergillus sp.* and *Alternaria sp.* Affected fruit show slightly abnormal skin color.

3. Objectives of Research/ Proposed Hypothesis:

1. To survey fruits cultivated in Marathwada region for post-harvest diseases.
2. To collect infected fruits of different varieties of different fruit.
3. To Isolate Post-harvest pathogens from selected fruits.
4. To identify microorganisms associated with post-harvest diseases by biochemical, morphological and molecular methods.
5. To screen native bio-control agents against selected post-harvest disease pathogens.
6. To assess effects of plant extracts against selected post-harvest disease pathogens.
7. Characterization of bioactive compounds from native bio-control agents and plant extracts using different analytical techniques.

4. Methodology to be adopted:

a. Isolation of bacterial and fungal pathogens of post harvest fruits :

Isolation of bacterial pathogens will be carried out by using the procedures described by Patel et al. (2015). Fruits showing typical symptoms of bacterial blight collected from the field and the bacterium will be isolated by dilution plate technique. Isolation of the pathogen is done within 48 hours after collection of samples on nutrient agar medium. Pure culture is established by repeated single colony transfer method.

The fungal pathogens will be isolated using the procedures described by Ilgın and Karac (2016). Plant samples with disease symptoms will be microscopically examined for fungal growth and structures under stereomicroscope. Samples with any fungal structure on them will be incubated on sterile blotter papers in petri dishes for

sporulation, in a climatic room with 22±2°C temperature, 12-12 hour of light and dark conditions and examined again.

Isolations will also be made by placing small pieces with diseased and healthy part of the plant tissue on PDA (Potato dextrose agar-Merck) and WA (Water agar 15-g agar, 1000 ml tap water) media, after surface disinfection with 1% NaOCl for 2-3 minutes. Three replicate plates will be used for each sample. Growing hyphal tips will be transferred to separate plates after 5-7 days incubation in the climatic room. Preparations will be made from the single colonies and fungi will be identified according to their cultural and microscopic features by using related. Isolates will be maintained on agar slants at 4°C temperature.

b. Molecular identification of bacterial and fungal pathogens of Post-harvest diseases of fruit:

The bacterial identification will be carried out by 16s rRNA sequencing and fungal identification will be carried out by 18s rRNA sequencing as per the procedures described by Rashid et al. (2016).

c. Isolation and characterization of native microorganism as bio-control agents

For isolation of fungal and bacterial strains, a serial dilution technique will be followed described by Rahman et al. 2009. Cultures will be identified and will be maintained for further study.

d. Extraction of root, stem, leaves and bark of some medicinal plants:

The extraction of root, stem, leaves and bark of locally available selected medicinal plants will be carried out using procedures described by Abah and Egwari (2011).

e. *In-vitro* analysis of antimicrobial activity of native microorganism as bio-control agents and extracts of selected plants:

The in-vitro analysis of antimicrobial activity of native microorganism as bio-control agents and extracts of selected plants against selected pathogens of some fruit will be as per the procedures described by Lu et al. (2004).

f. Characterization of antimicrobial compounds from bioactive extracts and native microorganism as bio-control agents:

The characterization of antimicrobial compounds from bioactive extracts of selected plants and of native microorganism as bio-control agents will be carried out by different analytical techniques such as FTIR, NMR, Mass Spectroscopy and HPLC analysis.

5.3 Significance of the study:

- The study will be helpful for the identification of different bacterial and fungal diseases of selected fruits.
- The study will be helpful for the isolation and activity characterization of *Trichoderma sp.* against selected pathogens.
- The study will be helpful for the identification effective plant extracts against selected post harvested diseases.
- Characterization of antimicrobial compounds from bioactive extracts of plants and *Trichoderma sp.* will be helpful for the identification of active compounds against the different post harvested diseases.
- The study will be helpful to increases life span of selected fruits as well as quality and market value.

6. Proposed work Plan/ Formulation and Structure of Study:

Year-wise Plan of work and targets to be achieved:

1. **First Year:**
   - Isolation of Post-harvest pathogens from selected fruits
   - Identification of bacterial and fungal pathogens by biochemical, morphological and molecular methods.
2. **Second Year:**
   - Isolation and activity characterization of native microorganism as bio-control agents against selected Post-harvest diseases of fruits .
   - Screening of root, stem, leaves and bark extracts of some medicinal plants (…) against selected bacterial and fungal pathogens.
3. **Third Year:**
   - Characterization of antimicrobial compounds from bioactive extracts of selected plant extracts and standardizing dose response curve
   - Analysis and compilation of data.

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